Study on Biofilm Formation in Burn Wound Infection in a Pediatric Hospital in Chennai, India

Ramakrishnan M.,1 Putli Bai S.,2 Babu M.3

1 Burns and Plastic Surgery Department, CHILDS Trust Medical Research Foundation, Chennai, India
2 Microbiology Department, Kanchi Kamakoti CHILDS Trust Hospital, Chennai, India
3 Research Department, CHILDS Trust Medical Research Foundation, Chennai, India

Summary: Infection is one of the major causes of death in pediatric burns in India. This work was conducted in an exclusive Children’s Hospital (KKCTH) with a total of 220 beds, of which ten beds in the burn unit and two isolation beds in the 28-bed PICU are for burns patients (more than 20% TBSA burns) with sepsis. In this study, 30 burn wound swab isolates obtained from 14 pediatric burns patients (admitted to the burns ward and transferred to PICU) from November 2013 to March 2014 were investigated. Cultures were done on the first day for all patients and empirical antibiotic administration was started for those with septic burns (14 in total) with piperacillin-tazobactam and vancomycin. Antibiotics were changed according to antibiotic sensitivity reports. Cultures were repeated for culture positive cases on the fifth day. Further antibiotic treatment was based on this culture report. When the general condition of the patient did not respond to high-level antibiotics, biofilm formation was suspected and evaluated as the possible cause of antibiotic resistance. For these patients, an enhanced method of wound debridement and albumin transfusions were used to improve their general condition. Microbial identification and antimicrobial sensitivity testing was done for all 30 isolates. The predominant bacteria were Pseudomonas aeruginosa, Acinetobacter and Staphylococcus aureus. Most of the Acinetobacter and Staphylococcus aureus showed multidrug resistance. Biofilm formation was studied using the Tissue Culture Plate (TCP) method for all bacterial isolates, and results showed that most of the MDR isolates formed biofilm.

Keywords: biofilm formation, burn sepsis, policy, antibiotics usage, burns

Introduction

Infection is a major cause of morbidity and mortality in burn patients.1,2 Three fourths of deaths in these patients occur due to infection. Burn patients are ideal hosts for opportunistic infections. The surface of every burn wound is contaminated to some degree with bacteria. Staphylococcus species and Pseudomonas aeruginosa are two of the most common micro flora that colonize burn wounds across the world.3 Ability to form biofilm on polymeric surfaces acts as an important pathogenic factor of bacteria during burn wound colonization. Biofilm consists of multilayered cell clusters embedded in a matrix of extracellular polysaccharide which facilitates adherence of these microorganisms to wound surfaces and protects them from host immune system and antibiotic therapy.

Biofilms are very difficult to eradicate and are commonly resistant to systemic antimicrobial therapy, which adds to the difficulty of burn wound management. The microbe’s ability to form biofilm can be detected in many ways: congo red agar (CRA), tube method (TM), tissue culture plate (TCP) and bioluminescent assay are some of the most common methods employed in routine labs. The TCP method (devised by Chris-
tensen et al.4) is one of the most widely used methods to phenotypically identify biofilm-producing strains. It uses 96-well plate reader spectrophotometer to measure the optical density of the formed biofilms.

This work was conducted in the Burn Unit and PICU of Kanchi Kamakoti CHILDs Trust Hospital, an exclusive Children’s Hospital in Chennai, India, with 220 beds. The unit has 10 beds for children between 0-18 years with burns and sepsis, who are transferred to the PICU. Twenty-eight beds are available in the PICU, and there are two isolation beds for patients with burns and sepsis.

Burn admissions are moved from the emergency room to the burns ward. Cases with 20-30% TBSA and over, deep partial thickness burns and deep burns with evidence of sepsis are transferred to the isolation beds in the PICU.

Table I and Fig. 1 show the number of burn patients admitted to the Burn Unit of Kanchi Kamakoti CHILDs Trust Hospital and the burns cases with sepsis treated in the PICU from November 2013 to March 2014. The septic burn cases transferred to the PICU between November 2013 and March 2014, which were 14 in total, were enrolled in this study.

The standard wound treatment adopted in the Burns Unit and in the PICU is the same. Closed dressings are applied over antibiotic tulle if the patients arrive with oozing wounds. Closed dressings are changed daily after sedating the children. If the burn wound is over 12 hours old and is dry without sepsis, it is covered with collagen dressing after cleaning, except when in the perineum.5 The collagen is kept in situ for 10-12 days if it adheres well. The wound surface swab is sent for culture and sensitivity as soon as patients arrive in the Burns Unit, as infection is always anticipated due to hot, humid weather conditions. Antibiotic prophylaxis is not usually given. Antibiotics are started if the culture report is positive. When the child becomes septic, he/she is transferred to the PICU.

Of the 36 patients admitted to the Burns Unit between November 2013 and March 2014, 14 were transferred to the PICU. These 14 patients were enrolled in this study.

We determined the prevalence of micro flora in the burn wounds of the 14 burn patients, screened the burn wound isolates for biofilm production using the TCP method, and compared the results with antibiotic resistance pattern.

Materials and methods

In this study, 30 burn wound swab isolates obtained from 14 pediatric burns patients (admitted to the burns ward and transferred to the PICU with over 20% burns) between November 2013 and March 2014 were investigated. Cultures were done on the first day for all patients, and empirical antibiotic administration was started with piperacillin-tazobactam and
vancomycin. Antibiotics were changed according to antibiotic sensitivity reports on the third day, and cultures were repeated on the fifth day. The antibiotics were stopped if the fifth-day culture report was negative. For fifth-day culture positive cases, antibiotics were enhanced and repeat culture was done on the tenth day. When the patient’s general condition did not respond to high-level antibiotics, biofilm formation was reviewed and evaluated as the possible cause of resistance to antibiotics.

Wound swabs were collected aseptically from burn wounds in the above 14 cases. The swabs were processed at the clinical microbiology lab of KKCTH. They were inoculated onto blood agar and MacConkey agar. The 30 wound swab isolates were identified using Gram stain and standard biochemical tests. Antimicrobial sensitivity tests were carried out using the Kirby-Bauer disc diffusion method. Biofilm formation capacity of the burn wound isolate was screened with the tissue culture plate method in the clinical microbiology lab of KKCTH. Antibiotic resistance pattern and biofilm formation capacity were compared.

**Biofilm detection**

The microtiter plate method\(^4\) was used to detect biofilm production. The isolated organism was inoculated in 10ml of trypticase soy broth (with 1% dextrose) and the broth was incubated at 37°C for 24 hrs. The incubated broth was diluted 1:100 with fresh trypticase soy broth (with 1% dextrose). 100µl of the broth culture was added to 10ml of fresh trypticase soy broth (with 1% dextrose) using sterile micropipette tip and mixed well. Each of the three individual wells of sterile 96-well flat bottom polystyrene tissue culture treated plates was inoculated with 200µl of the diluted broth culture. For positive control, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853 were used and they were incubated, diluted and added to tissue culture plates similar to the test isolate.

For negative control, each of the three individual wells of the sterile 96-well flat bottom polystyrene tissue culture treated plates was inoculated with 200µl of the uninoculated sterile trypticase soy broth with 1% dextrose. The tissue culture plates were incubated at 37°C for 24 hrs.

After incubation, the contents of each well were removed by gentle tapping and the wells were washed four times with 200µl of phosphate buffer saline to remove free floating bacteria. After washing, 200µl of 2% sodium acetate was added to each well and incubated for 15 minutes to fix the biofilm. After fixing, the plate was emptied and air-dried. 200µl of 0.1% crystal violet was added to each well and incubated for 15 minutes to stain the fixed biofilm. The excess stain was removed by washing with deionized water and the plate was air-dried.

The optical densities (OD) of wells were obtained using ELISA reader (Biorad) at a wavelength of 570nm. The mean OD value of negative control (ODnc) was calculated. The cutoff value was calculated from the ODnc, using the formula:

\[
\text{ODco} = \text{ODnc} + 3 \times \text{Standard deviation (SD) of nc}
\]

Average OD value of the test organism wells was calculated. Based on the OD value, the test organisms were classified as in **Table II**.

Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter spp, coagulase negative Staphylococcus (CONS), E.coli Klebsiella spp and Enterobacter cloacae were isolated from the burn wound swabs.

Pseudomonas aeruginosa was the predominant isolate (33.3%) followed by Acinetobacter (23.3%) and Staphylococcus aureus (16.6%) (**Fig. 2**). 50% of Pseudomonas aeruginosa were multidrug resistant (resistant to Antipseudomonal fluroquinolones, Aminoglycosides and Antipseudomonal cephalosporins) and 80% of them were biofilm producers. 86% of the Acinetobacter isolates were multidrug resistant (resistant to all penicillins and cephalosporins, including inhibitor com-

<table>
<thead>
<tr>
<th>Test organism average OD value</th>
<th>Biofilm production</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ ODco</td>
<td>Non biofilm producer</td>
</tr>
<tr>
<td>&gt; ODco and ≤ 2x ODco</td>
<td>Weak biofilm producer</td>
</tr>
<tr>
<td>&gt; 2x ODco and ≤ 4x ODco</td>
<td>Moderate biofilm producer</td>
</tr>
<tr>
<td>&gt; 4x ODco</td>
<td>Strong biofilm producer</td>
</tr>
</tbody>
</table>

**Table II - Classification of organism based on biofilm production**

<table>
<thead>
<tr>
<th>Patient outcome</th>
<th>Patient 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 culture isolates</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Day 5 culture isolates</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Day 10 culture isolates</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>13</td>
</tr>
</tbody>
</table>

**Fig. 2 - Burn wound isolates.**

<table>
<thead>
<tr>
<th>Strong/ moderate biofilm</th>
<th>Weak/ non biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

**Fig. 3 - Classification of biofilm producers.**

**Table III - Details of burn wound culture isolates**

<table>
<thead>
<tr>
<th>Patient outcome</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S: Survived</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>D</td>
<td>S</td>
<td>D</td>
<td>S</td>
<td>S</td>
<td>D</td>
<td>S</td>
<td>D</td>
<td>S</td>
<td>S: 10</td>
<td></td>
</tr>
<tr>
<td>D: Died</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D: 4</td>
<td></td>
</tr>
</tbody>
</table>
Bacterial infection is one of the main causes of mortality in burns patients. Good infection control practices play a major role in reducing these infections and thus reduce patient mortality and morbidity as well as treatment costs.

In our study, the isolates obtained from the four patients who died showed strong biofilm formation. A few of the isolates from burns patients who survived also showed biofilm formation, but their survival may be attributed to TBSA burned percentage and depth of burn wounds (deep partial thickness and not deep).

In our study, *Pseudomonas aeruginosa* was the most common burn wound isolate (33.3%), followed by *Acinetobacter* (23.3%) and *Staphylococcus aureus* (16.6%). Singh et al. and Patil et al. also found *Pseudomonas aeruginosa* to be the most commonly isolated organism from burns patients. Various studies show *Acinetobacter* to be one of the emerging pathogens in burn wound patients.

In our study, 50% of *Pseudomonas aeruginosa*, 86% of *Acinetobacter* and 75% of *Staphylococcus aureus* were multidrug resistant. A similar alarming trend was seen by Agnihotri et al. for both the Enterobacteriaceae group and for *Pseudomonas* species. Several studies have also shown an increasing prevalence of MRSA in burn infections.

Such high antimicrobial resistance among burn pathogens is probably due to selective pressure exerted on them by indiscriminate use of broad-spectrum antibiotics supported by ineffective hospital antibiotic policy. The multidrug resistant organisms establish themselves in the hospital environment and are transferred to the patient usually through contact. Infections by such multidrug resistant pathogens may be prevented by stringent infection control practices along with strict implementation of a hospital antibiotic policy.

Treatment of burn infections is challenging due to the pathogen’s antimicrobial resistance and its ability to form biofilm. Attachment of the microbe to surfaces and the formation of multicellular communities are considered to be important virulence factors. Various methods are available to detect the ability of the pathogen to produce biofilm, but the most widely used assay for evaluation of biofilm formation is the microtiter plate method.

In our study, the majority (80%) of *Staphylococcus aureus* produced biofilm, and most (75%) of the biofilm-producing *Staphylococcus aureus* isolates were Methicillin resistant (MRSA). Similar results were presented by Ohadian Moghadam et al.

Our results demonstrated that 44% of *Pseudomonas aeruginosa* were biofilm producers. Similar results were shown in other studies. More than half of the *Acinetobacter* isolates (57%) produced biofilm and most of them (67%) were multidrug resistant. Jayachandran et al. demonstrated that 62% of *A. baumannii* were biofilm positive. Shiva et al. showed that biofilm production occurred in 61.7% of *Acinetobacter* isolates.

Burn wound infection isolates that were resistant to multiple antibiotics were mostly biofilm producers, indicating that the majority of MDR pathogens are biofilm producers. A correlation was observed with other studies. Greatly enhanced tolerance to antibiotics is a characteristic of bacteria present in biofilms, which makes the management of burns infection a difficult task. Implementation of good infection control practices in burn centres plays a major role in the prevention of infection due to MDR pathogens.

---

**BIBLIOGRAPHY**


Acknowledgements: This research was supported by CHILDS Trust Medical Research Foundation (CTMRF), Kanchi Kamakoti CHILDS Trust Hospital, Chennai, India. We would like to thank the staff of the PICU for their continued support in this study.